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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant:	Ralph A. Nixon et al.	Art Unit:	1632
Serial No.:	09/560,124	Examiner:	Anne-Marie Falk
Filed:	April 28, 2000	Customer No.:	21559
Title:	METHODS FOR THE IDENTIFICATION OF COMPOUNDS FOR THE TREATMENT OF ALZHEIMER'S DISEASE		

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

DECLARATION OF RALPH NIXON, M.D., PH.D., UNDER 37 C.F.R. § 1.132 TRAVERSING GROUNDS OF REJECTION

Under 37 C.F.R. §1.132, I declare:

1. I am a Professor in the departments of Psychiatry and Cell Biology, New York University School of Medicine, and Director of Research, Nathan S. Kline Institute for Psychiatric Research, and an inventor on the above-captioned patent application. I have been studying Alzheimer's Disease for since 1970 and I have published more than 160 papers in the field.

2. I have read and understood the Office Action dated April 9, 2003.

3. The experiments carried out in the Declaration filed on January 8, 2003, were carried out using methods that were standard in the art at the time the application was filed.

4. As described in the specification and in paragraph 5, I, along with the other named inventors, have directed and conducted experiments demonstrating that cells with enlarged endosomes also have increased endocytic pathway activity, which is characterized by specific endosomal changes (e.g., increased endosomal fusion, endosomal recycling, expression of MPR46, accumulation of lysosomal hydrolases in early endosomes, and accumulation of Aβ in early endosomes) indicative of cells that are destined to be diseased in patients having Alzheimer's disease. rab5 overexpressing cells *in vitro* and *in vivo* mimic the increased endocytic pathway activity that is observed in patients with early stage Alzheimer's disease. Such compounds are excellent potential therapeutics given that they are likely to be useful in treating patients at a time when patients are essentially asymptomatic.

5. We have found that stably transfected murine L cells that overexpressed rab5 showed enlarged endosomes, similar to those observed in neurons from individuals with sporadic Alzheimer's Disease. In addition, a cDNA encoding the GTP-hydrolysis deficient rab5 mutant Q79L (Stenmark et al., EMBO J. 13:1287-1296, 1994) was expressed in L cells. Expression of the rab5 Q79L mutant resulted in increased endocytosis and the fusion of early endosomes into large vacuoles. Confirming that rab5 expression led to abnormal activity of the endosomal pathway, we also demonstrated both increased uptake of fluid phase markers (FITC-dextran) and increased receptor mediated endocytosis (transferrin).

6. As described in paragraph 7, experts accept that the presence of enlarged endosomes correlates with other markers of endosomal change, including endosomal fusion as evidenced in Roberts et al. (J. Cell Science 112:3667-3675, 1999).

7. Roberts et al. (J. Cell Science 112:3667-3675, 1999, hereafter "Roberts") show that the presence of enlarged endosomes correlates with endosomal fusion in cultured cells overexpressing rab5. Roberts overexpressed either wild-type rab5 or rab5:Q79L, a constitutively active rab5 mutant, in CHO and BHK cells and observed that the cells formed enlarged cytoplasmic vesicles that exhibited the characteristics of early endosomes. Using time-lapse video microscopy, Roberts showed that the enlarged endosomes resulted from endosomal fusion (page 3667, left column, abstract). Regarding these results, Roberts states, "Time-lapse video microscopy shows the enlarged endosomes arise primarily by fusion of smaller vesicles. These fusion events occur mostly by a "bridge' fusion mechanism in which the initial opening between vesicles does not expand; instead, membrane flows slowly and continuously from the smaller to the larger endosome in the fusing pair. . ."

8. In sum, we have shown and experts accept that cells having enlarged endosomes also exhibit increased endocytic pathway activity, which is characterized by specific endosomal changes (e.g., increased endosomal fusion, endosomal recycling, expression of MPR46, accumulation of lysosomal hydrolases in early endosomes, and accumulation of Aβ in early endosomes.)

9. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that willful false statements may jeopardize the validity of the application of any patents issued thereon.

Date: _____ _____

Dr. Ralph Nixon